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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Stephen D.	Gillies	Carlisle

TITLE OF THE INVENTION (280 characters max)

Targeted IL-12 Fusion Proteins

ENCLOSED APPLICATION PARTS *(check all that apply)*

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CORRESPONDENCE ADDRESS	SIGNATURE BLOCK
Direct all correspondence to: Patent Administrator Testa, Hurwitz & Thibeault, LLP High Street Tower 125 High Street Boston, MA 02110 Tel. No.: (617) 248-7000 Fax No.: (617) 248-7100 Customer No. 021323	Respectfully submitted, Date: January 5, 2004 Reg. No. 48,645 Tel. No.: (617) 248-7697 Fax No.: (617) 248-7100 Brian A. Fairchild Attorney for Applicant(s) Testa, Hurwitz & Thibeault, LLP High Street Tower 125 High Street Boston, MA 02110

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Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	US
Status::	
Given Name::	Stephen D.
Middle Name::	
Family Name::	Gillies
Name Suffix::	
City of Residence::	Carlisle
State or Province of Residence::	MA
Country of Residence::	US

Street of Mailing Address:: 47 Swanson Lane
City of Mailing Address:: Carlisle
State or Province of Mailing Address:: MA
Country of Mailing Address:: US
Postal or Zip Code of Mailing Address:: 01741

Correspondence Information

Correspondence Customer Number:: 021323

Representative Information

Representative Customer Number:: 021323

Express Mail Mailing Label No. EL932952149US

Targeted IL-12 Fusion Proteins

Abstract

Disclosed are fusion proteins that include an intact antibody directed against an antigen specific to tumor neovasculature, fused to interleukin-12. The fusion proteins are useful for treating solid tumors. The fusion proteins bind particularly tightly to the target antigen.

Background

Treatment of cancer with targeted fusion proteins has shown much promise, but many problems remain. For example, antibody-targeted cytokines have shown much promise in the treatment of cancer in animal models and in some human studies, but the optimal choice of antibody/antigen, cytokine, and antibody effector function remains to be determined. For example, Gillies (US5,650,150) described the general usefulness of cytokine fusions to complete antibodies, and the specific usefulness of antibody-IL2 fusion proteins.

Interleukin-12 (IL-12) is a particularly attractive cytokine for targeted immune therapy, because IL-12 stimulates a Th1 immune response, which is most effective in attacking tumor cells. IL-12 is quite toxic when administered systemically, so it is particularly important to direct its activity to a tumor site. Gillies *et al.* (WO99/29732) described the usefulness of fusions of IL-12 to antibodies, and also described particular techniques needed to express IL-12 fusion proteins, relating to the fact that IL-12 is a two-subunit cytokine in which one of the subunits can homodimerize. Halin *et al.* (Nature Biotechnology (2002), 20:264-269) described a fusion protein consisting of a single-chain IL-12 moiety fused to a single-chain Fv (sFv) with the variable domains of L19, an antibody that binds to tumor-specific neovasculature. This latter molecule lacks the Fc region of the antibody and thus lacks all effector functions.

Even when IL-12 is fused to a targeting moiety, there is a period after the fusion protein is administered when the protein drug circulates systemically. During this period, before the drug accumulates in the tumor and disappears from the rest of the system, secondary cytokines are induced and damage results. It would therefore be particularly useful that the binding of an antibody fusion protein to its target site be particularly tight. Thus, there is a need in the art for improved antibody-IL-12 fusion proteins.

Summary of the invention

The invention provides an immunoglobulin (Ig) fusion protein comprising antibody V regions that bind to oncofetal fibronectin, fused to interleukin-12. In a preferred embodiment of this invention, the antibody V regions are from the BC1 antibody (Carnemolla *et al.* (1992), J. Biol. Chem. 267:24689-24692; Mariani *et al.* (1997), Cancer 80:2378-2384). An unexpected feature of this invention is that the fusion protein binds to oncofetal fibronectin much more tightly than does the corresponding BC1 antibody alone. Such tight binding is useful in treating cancer, as the tighter binding leads to better tumor targeting of IL-12 than would be expected on the basis of the

affinity of the BC1 antibody for oncofetal fibronectin. Tighter binding is particularly advantageous for target antigens that do not turn over rapidly, such as components of the extracellular matrix.

In a preferred embodiment, antibody constant regions are also used. Figure 1 illustrates some of the configurations of antibody variable regions (striped ovals), constant regions (white ovals), the IL-12 p35 subunit (small rectangles), the IL-12 p40 subunit (large rectangles), antibody hinges and linkers (thick lines) and disulfide bonds (thin lines). Particular preferred embodiments include intact IgG-type antibodies with p35 fused to the C-terminus of the heavy chain and p40 attached to p35 by a disulfide bond (Figure 1A), a 'minibody' with the antibody V regions connected by a linker and attached through a hinge to a CH3 domain, and p35 fused to the C-terminus of the heavy chain and p40 attached to p35 by a disulfide bond (Figure 1B), an sFv with p35 fused to a V region and p40 attached by a disulfide bond (Figure 1C), and an Fab with p35 fused to a C region and p40 attached by a disulfide bond (Figure 1D). The IL-12 p35 subunit may also be attached to the N-terminus of a V region. The IL-12 p40 subunit may be attached to p35 through a disulfide bond or through a linker, yielding a so-called 'single-chain IL-12' moiety (scIL-12).

In a more preferred embodiment, an intact BC1 antibody with constant regions of human IgG1 is used. A particular advantage of this molecule is that is has effector functions such as ADCC, which are lacking in minibody, Fab, and sFv fusion proteins.

Detailed Description of the Invention

Interleukin-12 is considered to be a cytokine with great promise in the treatment of solid tumors. However, when IL-12 is administered systemically, secondary cytokines such as interferon-gamma are produced and toxic side effects result. It is therefore thought that the localization of IL-12 to a tumor site would be particularly useful.

Oncofetal fibronectin is a protein that is expressed by tumor cells and is associated with tumor vasculature. This protein is also expressed in fetal tissue, but does not appear to be expressed at all in normal adult tissue except for regenerating endometrium. Oncofetal fibronectin is generated by alternate splicing in tumor cells, through which an additional domain, the ED-B domain, is inserted between fibronectin repeats 7 and 8. The BC1 antibody specifically binds to oncofetal fibronectin by binding to a site on repeat 7 that is masked in normal fibronectin but accessible when the ED-B domain is present (Carnemolla *et al.* (1992), J. Biol. Chem. 267:24689-24692; Mariani *et al.* (1997), Cancer 80:2378-2384).

A BC1-IL12 fusion protein was constructed following the procedures of Gillies *et al.* (WO99/29732, incorporated herein by reference). In the course of characterizing the binding of the BC1-IL12 fusion protein to its target antigen, it was found that this fusion protein bound more tightly to its target than did the corresponding BC1 antibody itself. For example, the binding of BC1 and BC1-IL12 to a polypeptide including human fibronection domains 7, ED-B, 8, and 9 was measured using surface plasmon resonance. Table 1 summarizes the results of two experiments.

Table 1.

	muBC1 (murine constant regions)	HuBC1 (human constant regions)	huBC1-IL12
On-rate (1/mole/sec)	$1.65x10^3$ (exp. 2)	$6.2x10^4$ (exp.1) $7.3x10^4$ (exp.2)	$1.7x10^4$ (exp.1) $1.9x10^4$ (exp.2)
Off-rate (1/sec)	$1.1x10^{-3}$ (exp. 2)	$7.8x10^{-3}$ (exp.1) $1.0x10^{-2}$ (exp.2)	$1.3x10^{-3}$ (exp.1) $1.6x10^{-3}$ (exp.2)
Dissociation constant (nM)	686	125 (exp. 1) 138 (exp. 2)	7.6 (exp. 1) 8.3 (exp. 2)

The results indicate that the binding of huBC1-IL12 to its target antigen is at least 10-fold tighter, and most likely about 16-fold tighter, than the corresponding huBC1 antibody alone.

To confirm the results of the surface plasmon resonance study, U87 MG subcutaneous tumors were generated in immunocompromised SCID CB17 mice according to standard procedures, and tumor sections were immuno-stained with the huBC1 antibody and the huBC1-IL12 fusion protein. It was found that the intensity of staining with the huBC1-IL12 fusion protein was much greater than with the huBC1 antibody.

To verify the utility of BC1-IL12 fusion proteins in treatment of cancer, an huBC1-muIL12 fusion protein was constructed and expressed according to standard procedures (Gillies *et al.*, WO99/29732, incorporated herein by reference). This protein used murine IL-12 because human IL-12 is not recognized by murine IL-12 receptors.

SCID CB17 mice bearing U87MG glioblastoma tumors with a volume of about 140 cubic millimeters were treated with either huBC1 or huBC1-IL12 as shown in Table 2.

Table 2.

Protein	Dose regimen	Tumor volume at day 8	Tumor volume at day 13
huBC1-IL12	20 mcg, day 0-7	85	60
huBC1-IL12	5 mcg, day 0-7	130	120
huBC1-IL12	5 mcg, day 0, 2, 4, 6, 10, 12	115	70
huBC1	400 mcg, day 0, 4	170	175
- (PBS)	Day 0-7	180	195

huBC1-huIL12 is best produced in mammalian cells, such as NS/0 cells, according to standard procedures, such as those described in Gillies *et al.*, (WO99/29732). Figure 2 shows the amino acid sequences of the three mature polypeptides of huBC1-huIL12. To produce huBC1-IL12, nucleic acids encoding these protein sequences are placed in expression vectors according to standard techniques.

The huBC1-huIL12 protein may be formulated in PBS, in buffers containing arginine, citrate, mannitol, and/or Tween, or other standard protein formulation agents.

A protein of the invention may, for example, be purified using, in sequence, some or all of the following steps: Abx Mixed Resin column chromatography, recombinant Protein A chromatography, and Q Sepharose column chromatography, followed by Pellicon 2 tangential flow diafiltration for buffer exchange into formulation buffer. Virus inactivation and removal steps are interdigitated into these steps. The virus inactivation and removal steps are not necessary for purification per se, but are used to satisfy regulatory considerations.

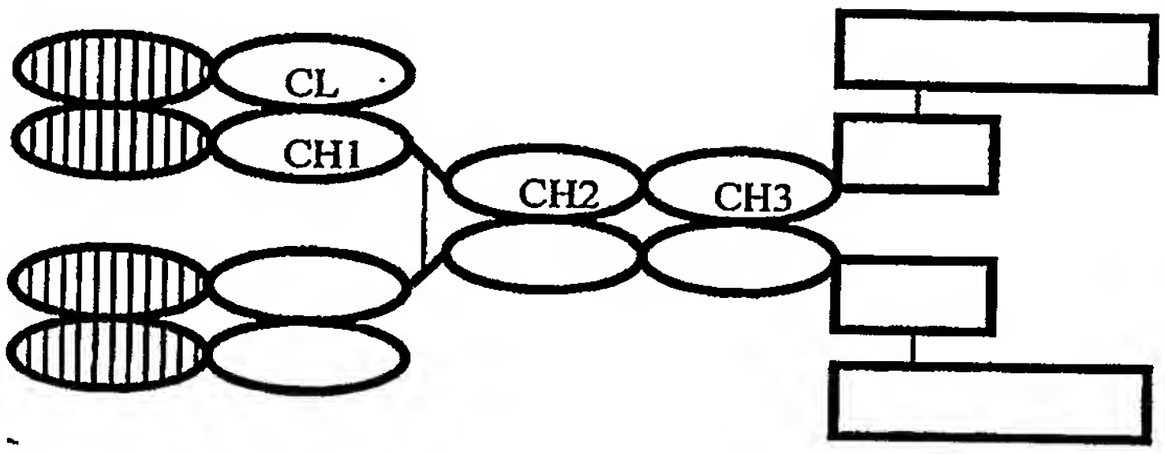
The BC1-IL12 fusion proteins of the invention are used as follows. A patient suffering from a cancer, such as glioblastoma, is treated. The preferred route of administration is intravenous or subcutaneous injection, but intramuscular, intraperitoneal, intradermal, or other routes of injection are also possible. Administration by inhalation, orally, or by suppositories is also possible, as are other routes of administration. Administration is preferably in a four-week cycle of three times per week, followed by no treatment for the next three weeks, but may be more or less frequent depending on the pharmacokinetic behavior of the BC1-IL12 protein in a given individual. Dosing for an adult of about 70 kilograms is in the range of about 1 to 100 milligrams per dose, with a preferred range of about 4 to 20 milligrams per dose. The most preferred dose is about 10 milligrams for a 70 kg adult treated once per month. Patients are monitored for a response according to standard procedures.

Claims

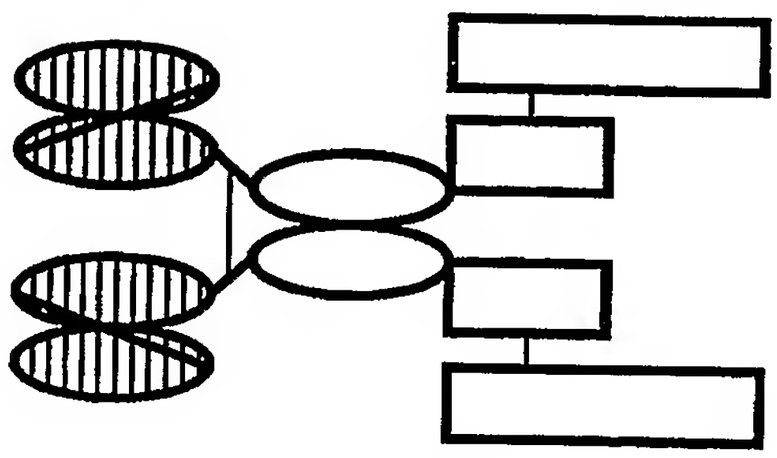
1. A fusion protein comprising: antibody V regions directed against oncofetal fibronectin, an Fc moiety, and an interleukin-12 moiety.
2. The fusion protein of claim 1, comprising a CH1 domain.
3. The fusion protein of claim 1, where the Fc moiety is derived from human IgG1.
4. The fusion protein of claim 1, where the binding of the fusion protein to the target antigen is tighter than the binding of the corresponding protein lacking the interleukin-12 moiety to the target antigen.
5. A fusion protein comprising antibody V regions as shown in SEQ ID 1 and 2, and an interleukin-12 moiety.
6. A fusion protein of claim 5, where the Fc moiety is derived from human IgG1.
7. A protein of any of claims 1 through 6, wherein said IL-12 moiety is a single-chain interleukin-12.

Figure 1. BC1-IL12 configurations.

A.



B.



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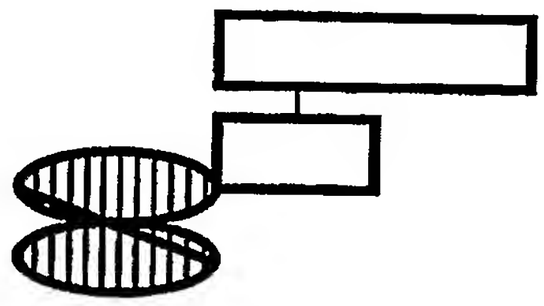

D.

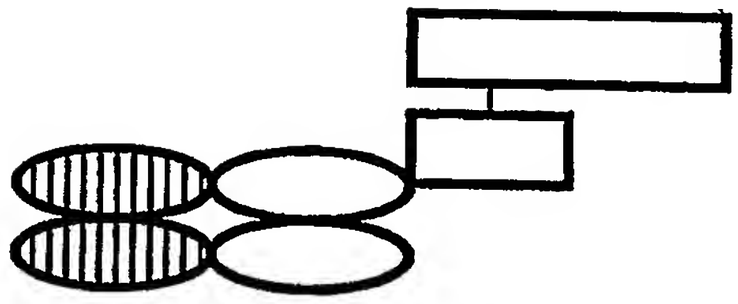

Figure 2. Amino acid sequences of the invention

BC1 heavy chain fused to human IL-12 p35

EVQLVQSGADVKKPGASVKVSCKASGYTFTNYVMHWVRQAPGQGLEWLGYIN
PYNDGTQYNERFKGRVTMTGDTSISTAYMELSRLTSDDTAVYYCAREVYGNYI
WGNWGQGTLVSVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSATATPGAANLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQ
TLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASR
KTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELM
QALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

BC1 light chain

EIVLTQSPGTLSLSPGERATLSCSASSSISSNYLHWYQQKPGQAPRLLIYRTSNLAS
GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGSSIPFTFGQGTKLEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Human IL-12 p40

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLT
IQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLR
CEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDN
KEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN
LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKT
SATVICRKNASISVRAQDRYYSSSWSEWASVPCS